TABLE OF CONTENTS

1. INTRODUCTION 3

1.1 Diabetes Mellitus 3

1.1.1 TREATMENT OF DIABETES MELLITUS 4

1.2 Stability indicating method 7

1.3 Degradation kinetic study 7

2. REVIEW OF LITERATURE 8

2.1 Drug profile 8

2.1.1 Repaglinide **(CAS- 135062-02-1)** 8

2.1.2 Sitagliptin Phosphate **(CAS- 486460-32-6)** 10

2.2 Official methods of analysis 12

2.2.1 Repaglinide 12

2.2.2 Sitagliptin phosphate 12

2.3 Reported methods of analysis 12

2.3.1 Repaglinide 12

2.3.2 Sitagliptin Phosphate 14

3. AIM OF WORK 18

4. EXPERIMENTAL 19

4.1 Instruments and Apparatus 19

4.2 Reagents and Materials 19

4.3 Method development and validation for Repaglinide 20

4.3.1 HPTLC method 20

4.3.2 HPLC method 21

4.4 Method development and validation for Sitagliptin phosphate 22

4.4.1 HPTLC method 22

4.4.2 HPLC method 23

4.5 Isolation and characterization of degradation products 25
4.5.1 Repaglinide 25
4.5.2 Sitagliptin phosphate 25
4.6 Degradation kinetic study of sitagliptin phosphate in alkaline medium 25
5. RESULTS AND DISCUSSION 26
5.1 Method development and validation for Repaglinide 26
5.1.1 HPTLC method 26
5.1.2 HPLC method 27
5.2 Method development and validation for Sitagliptin phosphate 28
5.2.1 HPTLC method 28
5.2.2 HPLC method 29
5.3 Isolation and characterization of degradation products 30
5.3.1 Repaglinide 30
5.3.2 Sitagliptin phosphate 30
5.4 Degradation kinetics study of Sitagliptin phosphate in alkaline medium 30
6. CONCLUSION 30
7. REFERENCES 31

1. INTRODUCTION

1.1 DIABETES MELLITUS

Diabetes mellitus is a chronic metabolic disorder characterised by a high blood glucose concentration-hyperglycaemia (fasting plasma glucose > 7.0 mmol/L, or plasma glucose > 11.1 mmol/L 2 hours after a meal)-caused by insulin deficiency, often combined with insulin resistance. Hyperglycaemia occurs because of uncontrolled hepatic glucose output and reduced uptake of glucose by skeletal muscle with reduced glycogen synthesis. When the renal threshold for glucose reabsorption is exceeded, glucose spills over into the urine (glycosuria) and causes an osmotic diuresis (polyuria), which, in turn, results in dehydration, thirst and increased drinking (polydipsia). Insulin deficiency causes wasting through increased breakdown and reduced synthesis of proteins. Diabetic ketoacidosis is an acute emergency. It develops in the absence of insulin because of accelerated breakdown of fat to acetyl-CoA, which, in the absence of aerobic carbohydrate metabolism, is converted to acetoacetate and β-hydroxybutyrate (which cause acidosis) and acetone.[1,2,3]

Various complications develop as a consequence of the metabolic derangements in diabetes, often over many years. Many of these are the result of disease of blood vessels, either large (macrovascular disease) or small (microangiopathy). Dysfunction of vascular endothelium is an early and critical event in the development of vascular complications. Oxygen-derived free radicals, protein kinase C and non-enzymic products of glucose and albumin (called advanced glycation end products) have been implicated. Macrovascular disease consists of accelerated atheroma and its thrombotic complications, which are commoner and more severe in diabetic patients. Microangiopathy is a distinctive feature of diabetes mellitus and particularly affects the retina, kidney and peripheral nerves. Diabetes mellitus is the commonest cause of chronic renal failure, which itself represents a huge and rapidly increasing problem, the costs of which to society as well as to individual patients are staggering. Coexistent hypertension promotes progressive renal damage, and treatment of hypertension slows the progression of diabetic nephropathy and reduces myocardial infarction. Angiotensin-converting enzyme inhibitors or angiotensin receptor antagonists are more effective in preventing diabetic nephropathy than other antihypertensive drugs, perhaps because they prevent fibroproliferative actions of angiotensin II and aldosterone.[1,2,3]

The disease states underlying the diagnosis of diabetes mellitus are now classified into four categories:[4]

Type 1, Insulin-Dependent Diabetes Mellitus (IDDM)

Type 2, Non Insulin-Dependent Diabetes Mellitus (NIDDM)

Type 3, other

Type 4, Gestational Diabetes Mellitus (Expert Committee 2002, Mayfield, 1998)

1.1.1 TREATMENT OF DIABETES MELLITUS

1.1.1.1 INSULIN THERAPY

Insulin is the mainstay for treatment of virtually all type 1 DM and many type 2 DM patients. When necessary, insulin may be administered intravenously or intramuscularly; however, long-term treatment relies predominantly on subcutaneous injection of the hormone. Subcutaneous administration of insulin differs from physiological secretion of insulin in at least two major ways: The kinetics do not reproduce the normal rapid rise and decline of insulin secretion in response to ingestion of nutrients, and the insulin diffuses into the peripheral circulation instead of being released into the portal circulation; the direct effect of secreted insulin on hepatic metabolic processes thus is eliminated. Nonetheless, when such treatment is performed carefully, considerable success is achieved.[1,2]

Preparations of insulin can be classified according to their duration of action into short, intermediate, and long acting and by their species of origin, human or porcine. Human insulin (HUMULIN, NOVOLIN) is now widely available as a result of its recombinant production. Porcine insulin differs from human insulin by one amino acid (alanine instead of threonine at the carboxy terminal of the B chain, i.e., in position B30. Prior to the mid-1970s, commercially available insulin preparations contained proinsulin or glucagonlike substances, pancreatic polypeptide, somatostatin, and vasoactive intestinal peptides. These contaminants were avoided with the advent of monocomponent porcine insulins. During the late 1970s, intense work was carried out on the development of biosynthetic human insulin. During the last decade, human insulin rapidly has become the standard form of therapy, and beef insulin products have been discontinued in the United States.[1,2]

1.1.1.2 ORAL HYPOGLYCEMIC AGENTS

Insulin Secretagogues

Sulfonylureas [1,3]

The sulfonylureas were developed following the chance observation that a sulfonamide derivative (used to treat typhoid) caused hypoglycaemia. They are divided into two groups or generations of agents. All members of this class of drugs are substituted arylsulfonylureas. They differ by substitutions at the para position on the benzene ring and at one nitrogen residue of the urea moiety. The first group of sulfonylureas includes tolbutamide, acetohexamide, tolazamide, and chlorpropamide. A second, more potent generation of hypoglycemic sulfonylureas has emerged, including glyburide (glibenclamide), glipizide, gliclazide, and glimepiride.

Meglitinides [1,3]

The meglitinides are a relatively new class of insulin secretagogues. Repaglinide, the first member of the group, was approved for clinical use in 1998. These drugs modulate B cell insulin release by regulating potassium efflux through the potassium channels. There is overlap with the sulfonylureas in their molecular sites of action since the meglitinides have two binding sites in common with the sulfonylureas and one unique binding site. Unlike the sulfonylureas, they have no direct effect on insulin exocytosis.

GLP-1 analogues [1, 5]

GLP-1 is a 30 amino acid peptide produced and secreted by the L cells of the intestinal mucosa in response to ingestion of carbohydrates and lipids. It stimulates β-cell activity via its Gs-coupled receptor, leading to stimulation of β-cell proliferation and differentiation, insulin gene expression and insulin secretion. Importantly, GLP-1 enhances insulin release in a glucose-dependent manner and therefore has less risk of causing hypoglycemia.

GLP-1 itself cannot practically be used as a treatment for diabetes. It has a plasma half-life less than a few minutes as it is degraded to its inactive form by DPP-IV and therefore requires multiple subcutaneous injections. GLP-1 analogues have therefore been developed to produce a longer terminal elimination half-life, and these have been shown to cause weight loss and improvement in glycemia in the diabetic population. It has been postulated that shorter acting GLP-1 analogues (exenatide and lixisenatide) reduce hyperglycemia primarily by slowing gastric emptying, whereas longer acting GLP-1 analogues (liraglutide, exenatide long-acting release, albiglutide and dulaglutide) predominantly lower postprandial glucose levels by insulinotropy and glucagon inhibition.

DPP-4 inhibitors [6]

The dipeptidyl peptidase-4 (DPP-4) inhibitors are a new class of oral drugs for the treatment of type 2 diabetes. They inhibit the breakdown of glucagon-like peptide-1 (GLP-1) and increase the incretin effect in patients with type 2 diabetes. In clinical practice they are associated with significant reductions in HbA1c, no weight gain and a low risk of hypoglycaemia. Initial cardiovascular safety studies have shown no increase in cardiovascular risk. Indeed, the suggestion of possible cardiovascular benefit seen in the safety studies is now being formally examined in large randomised-controlled trials with primary cardiovascular end points.

Examples: Sitagliptin, Saxagliptin, Alogliptin, Linagliptin

Insulin Sensitizers

Biguanides [1, 2]

Metformin and phenformin were introduced in 1957, and buformin was introduced in 1958. Phenformin was withdrawn in many countries during the 1970s because of an association with lactic acidosis. Metformin has been associated only rarely with that complication and has been used widely in Europe and Canada; it became available in the United States in 1995. Metformin given alone or in combination with a sulfonylurea improves glycemic control and lipid concentrations in patients who respond poorly to diet or to a sulfonylurea alone.

Thiazolidinediones[1, 2]

The thiazolidinediones (or glitazones) were developed following the chance observation that a clofibrate analogue, ciglitazone, which was being screened for effects on lipids, unexpectedly lowered blood glucose. Three thiazolidinediones have been used in clinical practice (troglitazone, rosiglitazone, and pioglitazone); however, ciglitazone and troglitazone were withdrawn from use because they were associated with severe hepatic toxicity. Rosiglitazone and pioglitazone can lower hemoglobin A1c levels by 1% to 1.5% in patients with type 2 DM. These drugs can be combined with insulin or other classes of oral glucose-lowering agents. The thiazolidinediones tend to increase high-density lipoprotein (HDL) cholesterol but have variable effects on triglycerides and low-density lipoprotein (LDL) cholesterol.

Dual PPAR agonists[1, 2]

Glitazars are dual peroxisome proliferator- activated receptors (PPAR) alpha/gamma agonists that improve the lipid profile and exert an antidiabetic action – similar to a combination of a fibrate and a thiazolidinedione. In May 2006 the two glitazars most advanced in development, muraglitazar (Pargluva) and tesaglitazar (Galida) were discontinued. Muraglitazar was associated with an increased incidence of heart failure and tesaglitazar was associated with decreased glomerular filtration.

In May 2006 development of two glitazar drugs was discontinued. These were muraglitazar (Pargluva, BMS/MSD) and tesaglitazar (Galida, AZ) both of which had recently completed phase III clinical trials.

Others

Alpha-Glucosidase inhibitors[1, 2]

α-Glucosidase inhibitors reduce intestinal absorption of starch, dextrin, and disaccharides by inhibiting the action of a-glucosidase in the intestinal brush border. Inhibition of this enzyme slows the absorption of carbohydrates; the postprandial rise in plasma glucose is blunted in both normal and diabetic subjects. α-Glucosidase inhibitors do not stimulate insulin release and therefore do not result in hypoglycemia. These agents may be considered as monotherapy in elderly patients or in patients with predominantly postprandial hyperglycemia. α-Glucosidase inhibitors typically are used in combination with other oral antidiabetic agents and/or insulin. The drugs should be administered at the start of a meal. They are poorly absorbed. Acarbose, an oligosaccharide of microbial origin, and miglitol, a desoxynojirimycin derivative, also competitively inhibit glucoamylase and sucrase but have weak effects on pancreatic a-amylase. They reduce postprandial plasma glucose levels in type 1 and type 2 DM subjects. α-Glucosidase inhibitors can significantly improve hemoglobin A1c levels in severely hyperglycemic type 2 DM patients. However, in patients with mild-to-moderate hyperglycemia, the glucose-lowering potential of α-glucosidase inhibitors (assessed by hemoglobin A1c levels) is about 30% to 50% of that of other oral antidiabetic agents.

1.2 STABILITY INDICATING METHOD

The accepted definition of a stability indicating method for a traditional (small molecules) pharmaceutical is a chromatographic (or other separation) method, able to separate the reportable degradants generated upon long-term storage of the product. Traditionally, the stability-indicating quality of the method is demonstrated by using stressed samples or long-term stability samples.[7]

1.3 DEGRADATION KINETIC STUDY

Kinetic principles are of great importance in stability study of dosage form. The study of drug degradation kinetics is of greater importance for development of stable formulation and establishment of expiration date for commercially available drug products, in laboratories of pharmaceutical industries. In spite of the importance of degradation kinetic for development of stable dosage form, there have been relatively few attempts to evaluate the detail kinetic of their decomposition. The degradation rate kinetic gives the information regarding the rate of process that generally leads to the inactivation of drug through either decomposition or loss of drug by conversion to a less favorable physical or chemical form. The kinetic and stability are not identical but they are different in following ways, chemical kinetic is studies through half-lives. Stability studies down up to 85% of the initial strength. Chemical kinetic is carried out in pure system, while stability study system contains relatively many components. The goal of chemical kinetic is to elucidate reaction mechanism, where as that of stability study is to establish expiration date.[7]

2. REVIEW OF LITERATURE

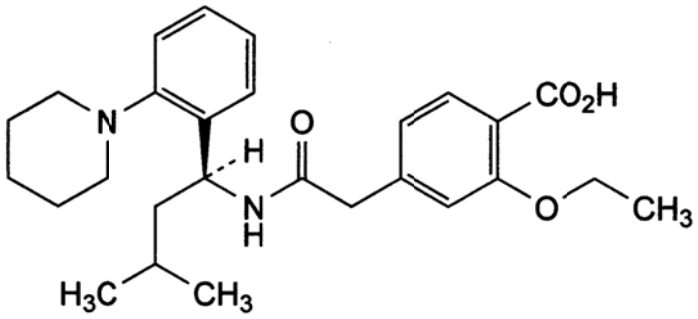
2.1 DRUG PROFILE

2.1.1 REPAGLINIDE **(CAS- 135062-02-1)**

Repaglinide is a nonsulfonylurea insulin secretagogue that was introduced in 1998 for type 2 diabetes in United States. This agent is a derivative of benzoic acid, and its structure is unrelated to that of the sulfonylureas. Repaglinide stimulates insulin release by closing ATP-dependent potassium channels in pancreatic β cells. It has a rapid onset and short duration of action compared to other hypoglycemic agents. It is not associated with the prolonged five hyperinsulinemia seen with sulfonylureas, and possibly for this reason, it produces fewer side effects, including weight gain and potentially dangerous hypoglycemia. Repaglinide is at least five fold more potent than glyburide on intravenous administration and nearly 10-fold more active on oral administration. Some of its physicochemical and pharmacokinetic parameters are described below.[3]

Physicochemical parameters[8,9, 10, 11]

Molecular structure:



Empirical Formula: $C_{27}H_{36}N_2O_4$

Molecular weight: 452.6; C-71.65%, H-8.02%, N-6.19%, O-14.14%

Chemical Name:

2-Ethoxy-4-[2-[[(1*S*)-3-methyl-1-[2-(piperidin-1-yl)phenyl]butyl]amino]-2 oxoethyl]benzoic acid;

(+)-2-ethoxy-α-[[(s)-α-isobutyl-o-piperidinobenzyl]carbamoyl]-p-toluic acid;

(s)-(+)-2-ethoxy-4[N-[1-(2-piperidinophenyl)-3-methyl-1-butyl]aminocarbonyl methyl]benzoic acid

Synonyms: AG-EE-338 ZW, AG-EE-623 ZW, AG-EE-6232W

Appearance: White or almost white, odourless, crystalline powder

Solubility: Practically insoluble in water, freely soluble in methanol and in methylene chloride. It shows polymorphism.

Melting point: 126° to 128°C for crystals from ethanol/water (2:1); 130° to 131°C for crystals from neutralized water

Specific optical rotation: + 6.3° to + 7.3° at 20°C; test solution: 50 mg/mL in methanol; +6.97° at 20°C (c = 0.975 in methanol) and +7.45° at 20°C (c = 1.06 in methanol)

LogP: 4.86

LogD: 2.10 at pH 7.0

pK_a: 4.19 (HA); 5.78 (BH^+)

Ultraviolet spectrum:

Infra-red spectrum: Principle peaks at wavenumbers 1688, 1636, 1215, 1588, 1150, 1607 cm^{-1}.

Mass Spectrum: Principle ions at *m/z* 409, 172, 452, 186, 245, 228, 130, 396.

Pharmacokinetic parameters [8, 9, 12, 13]

Absorption and distribution: Repaglinide is rapidly and completely absorbed after oral administration and peak concentrations are observed in approximately 1 h. The presence of food does not affect the absorption or pharmacokinetics of Repaglinide.

Metabolism: Plasma levels decrease rapidly once the peak concentration has been reached. It undergoes almost complete hepatic metabolism involving cytochrome P450 CYP3A4. Major metabolites include the oxidized carboxylic acid, aromatic amine and the acyl glucuronide, none of which are active with clinically relevant hypoglycaemic activity.

Elimination: The drug is eliminated rapidly, usually within 4 to 6 h, and excreted primarily via bile as both parent compound and its metabolized. Less than 8% of an administered dose is excreted in urine, mainly as the metabolites, and less than 1 % of the dose is detected in faeces as the unchanged drug.

Bioavailability: Approximately 63%

Half-life: 1 h

Clearance: Plasma, 143 mL/min

Volume of distribution: 20 to 30 L

Protein binding: Greater than 98%

Dose: the initial dose is 0.5 to 2.0 mg administered 30min before meals. 1.0mg or above is administered to patients who have had previous hypoglycaemic treatment. The dose is adjusted every 1 to 2 weeks up to 4.0 mg. A total daily dose of 16.0mg should not be exceeded.

LD_{50} (orally in rats): greater than 1 gm/kg

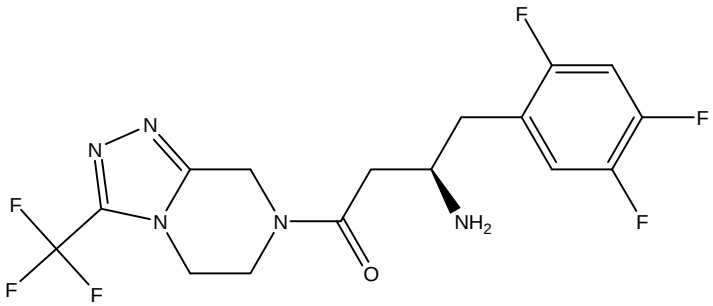
Therapeutic category: Hypoglycaemic, Antidiabetic

2.1.2 SITAGLIPTIN PHOSPHATE **(CAS- 486460-32-6)**

Sitagliptin is a DPP-4 inhibitor, which is believed to exert its actions in patients with type 2 diabetes by slowing the inactivation of incretin hormones. Concentrations of the active intact hormones are increased by Januvia, thereby increasing and prolonging the action of these hormones. Incretin hormones, including glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), are released by the intestine throughout the day, and levels are increased in response to a meal. These hormones are rapidly inactivated by the enzyme, DPP-4. The incretins are part of an endogenous system involved in the physiologic regulation of glucose homeostasis. When blood glucose concentrations are normal or elevated, GLP-1 and GIP increase insulin synthesis and release from pancreatic beta cells by intracellular signaling pathways involving cyclic AMP. GLP-1 also lowers glucagon secretion from pancreatic alpha cells, leading to reduced hepatic glucose production. By increasing and prolonging active incretin levels, Januvia increases insulin release and decreases glucagon levels in the circulation in a glucose-dependent manner. Sitagliptin demonstrates selectivity for DPP-4 and does not inhibit DPP-8 or DPP-9 activity in vitro at concentrations approximating those from therapeutic doses.

Physicochemical parameters [9, 14]

Molecular structure:



$H_3PO_4.H_2O$

Empirical Formula: $C_{16}H_{15}F_6N_5O.H_3PO_4.H_2O$

Molecular weight: 523.32

Chemical Name: (3R)-3-amino-1-[3-(trifluoromethyl)-6,8-dihydro-5H-[1,2,4]triazolo[4,3-a]pyrazin-7-yl]-4-(2,4,5-trifluorophenyl)butan-1-one

Appearance: White to off-white crystalline, non-hygroscopic powder

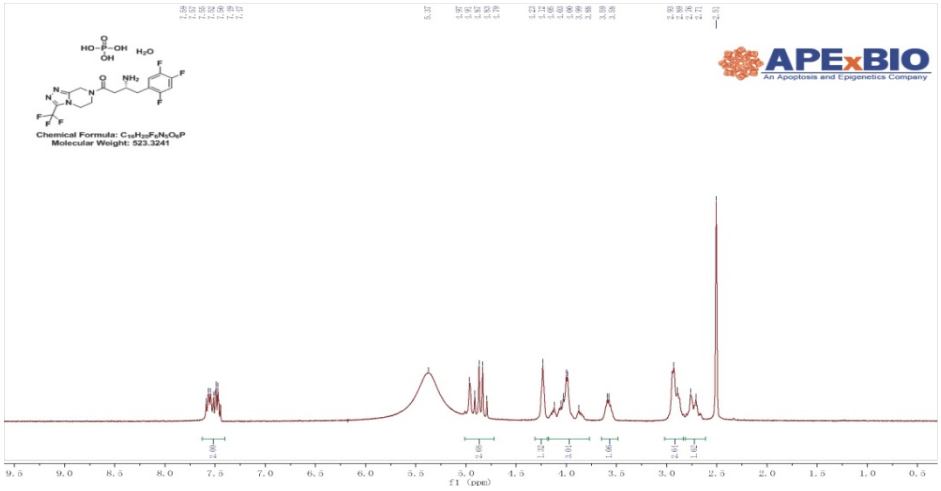
Solubility: Soluble in water and N,N-dimethyl formamide; slightly soluble in methanol; very slightly soluble in ethanol, acetone, and acetonitrile; and insoluble in isopropanol and isopropyl acetate

Melting point: 215-217° C

Specific optical rotation: -74.4° (C=1.0 in water)

LogP: 1.5

NMR spectrum:



Pharmacokinetic parameters

Absorption and distribution: Rapidly absorbed following oral administration, with an absolute bioavailability of 87%

Metabolism: Sitagliptin does not undergo extensive metabolism. In vitro studies indicate that the primary enzyme responsible for the limited metabolism of sitagliptin was CYP3A4 (oxidation), with contribution from CYP2C8.

Elimination: Approximately 79% of sitagliptin is excreted unchanged in the urine with metabolism being a minor pathway of elimination. Following administration of an oral [14C]sitagliptin dose to healthy subjects, approximately 100% of the administered radioactivity was eliminated in feces (13%) or urine (87%) within one week of dosing. Elimination of sitagliptin occurs primarily via renal excretion and involves active tubular secretion.

Half-life: 12.4 hours

Clearance: Renal clearance 350 mL/min

Volume of distribution: 198 L

Protein binding: The fraction of sitagliptin reversibly bound to plasma proteins is low (38%)

Dose: 100 mg orally once daily

Therapeutic category: Antidiabetic

2.2 OFFICIAL METHODS OF ANALYSIS

2.2.1 REPAGLINIDE

Repaglinide is not official in Indian Pharmacopoeia (IP) 2010 but it is official in British Pharmacopoeia (BP) 2007 and United States Pharmacopoeia (USP) 30-National Formulary (NF) 25. BP describes non-aqueous titrimetric method for the assay of Repaglinide using 0.1M perchloric acid as titrant, methanol and anhydrous acetic acid as solvents and determining the end point potentiometrically. Liquid chromatographic method has been used in BP for the test of related substances.[10,11]

USP describes liquid chromatographic method for assay of Repaglinide. USP utilizes similar method for testing chromatographic purity of Repaglinide with modifications in mobile phase and time program.[11]

2.2.2 SITAGLIPTIN PHOSPHATE

Sitagiptin phosphate is not official in IP 2010, BP 2007 or USP 30-NF 25.

2.3 REPORTED METHODS OF ANALYSIS

2.3.1 REPAGLINIDE

Several other analytical methods have been also reported in literature for determination of Repaglinide in bulk, pharmaceutical formulations and in biological fluids like human plasma. Some of them have been applied to pharmacokinetic study, dissolution testing, enantiomeric separation and other in-vitro analysis of Repaglinide. Table 1 gives an account of reported methods for analysis of repaglinide.

Table 1- Reported methods for analysis of repaglinide

Spectrophotometric and spectrofluorimetric methods		
Estimation of ripaglinide alone		
Sr. No.	**Method**	**Reference**
1.	Visible spectrophotometric methods for estimation of repaglinide in tablet formulation	15
2.	Development of Spectrofluorimetric and HPLC Methods for In vitro Analysis of Repaglinide	16
3.	Validated spectrophotometric methods for determination of some oral hypoglycemic drugs	17
4.	Comparison of UV spectrophotometry and high performance liquid chromatography methods for the determination of repaglinide in tablets	18
5.	Validated stability-indicating spectrofluorimetric method with enhanced sensitivity for determination of repaglinide in tablets.	19
6.	A comparative study of first-derivative spectrophotometry and column high-performance liquid chromatography applied to the determination of repaglinide in tablets and for dissolution testing	20

Simultaneous estimation of ripaglinide with other drugs		
Sr. No.	**Method**	**Reference**
1.	Spectrophotometric determination of repaglinide and ezetimibe	[21]
2.	Simultaneous spectrophotometric estimation of metformin and repaglinide in a synthetic mixture	[22]
3.	Development and validation of UV spectroscopic methods for the estimation of Repaglinide and Metformin hydrochloride in synthetic mixture	[23]
Chromatographic methods: HPLC & LC-MS methods		
Estimation of ripaglinide alone		
Sr. No.	**Method**	**Reference**
1.	Quantitation of the new hypoglycaemic agent AG-EE 388 ZW in human plasma by automated high-performance liquid chromatography with electrochemical detection	[24]
2.	Determination of Repaglinide in Pharmaceutical Formulations by HPLC method with UV detection	[25]
3.	Method development and validation of repaglinide in human plasma by HPLC and its application in pharmacokinetic studies	[26]
4.	Determination of Repaglinide in Pharmaceutical Formulations by RP-HPLC Method	[27]
5.	Stability Indicating RP-HPLC Method for Determination and Validation of Repaglinide in Pharmaceutical Dosage Form	[28]
6.	Determination of repaglinide in human plasma by high-performance liquid chromatography–tandem mass spectrometry	[28]
7.		
Simultaneous estimation of ripaglinide with other drugs		
Sr. No.	**Method**	**Reference**
1.	Detection of anti-diabetics in equine plasma and urine by liquid chromatography-tandem mass spectrometry	[29]
2.	Development and Validation of a New High-Performance Liquid Chromatography Method for the Determination of Gliclazide and Repaglinide in Pharmaceutical Formulations	[30]
3.	Simultaneous estimation of six anti-diabetic drugs--glibenclamide, gliclazide, glipizide, pioglitazone, repaglinide and rosiglitazone: development of a novel HPLC method for use in the analysis of pharmaceutical formulations and its application to human plasma assay	[31]
4.	Multi-component plasma quantitation of anti-hyperglycemic pharmaceutical compounds using liquid chromatography-tandem mass spectrometry	[32]
5.	Development of a RP-HPLC method for screening potentially counterfeit anti-diabetic drugs	[33]
6.	Analysis of nine drugs and their cytochrome P450-specific probe metabolites from urine by liquid chromatography-tandem mass spectrometry utilizing sub 2 microm particle size column	[34]
7.	Simultaneous identification and validated quantification of 11 oral hypoglycaemic drugs in plasma by electrospray ionisation liquid chromatography-mass spectrometry	[35]

8.	Separation and Quantification of Eight Antidiabetic Drugs on A High-Performance Liquid Chromatography: Its Application to Human Plasma Assay	[36]
9.	Development and validation of RP-HPLC method for simultaneous estimation of metformin hydrochloride and repaglinide in tablet dosage form	[37]
10.	Liquid chromatography-tandem mass spectrometry simultaneous determination of repaglinide and metformin in human plasma and its application to bioequivalence study	[38]
11.	LC-MS/MS-ESI method for simultaneous quantitation of metformin and repaglinidie in rat plasma and its application to pharmacokinetic study in rats.	[39]
Chromatographic methods: HPTLC methods		
Estimation of ripaglinide alone		
Sr. No.	**Method**	**Reference**
1.	Quantitative Analysis of Repaglinide in Tablets by Reversed-Phase Thin-Layer Chromatography with Densitometric UV Detection	[40]
2.	Estimation of repaglinide in bulk and tablet dosage forms by HPTLC method	[41]
Other methods		
Sr. No.	**Method**	**Reference**
1.	A Validated Chiral LC Method for the Enantiomeric Separation of Repaglinide on Amylose Based Stationary Phase	[42]
2.	Electrochemical Determination of the Antidiabetic Drug Repaglinide	[43]
3.	Determination of antihyperglycemic drugs in nanomolar concentration levels by micellar electrokinetic chromatography with non-ionic surfactant	[44]
4.	Electrochemical characterization of repaglinide and its determination in human plasma using liquid chromatography with dual-channel coulometric detection	[45]
5.	Analysis of repaglinide enantiomers in pharmaceutical formulations by capillary electrophoresis using 2,6-di-o-methyl-β-cyclodextrin as a chiral selector	[46]

2.3.2 SITAGLIPTIN PHOSPHATE

Several other analytical methods have been also reported in literature for determination of Sitaglptin in bulk, pharmaceutical formulations and in biological fluids.

Table 2- Reported methods for analysis of sitagliptin phosphate

Spectrophotometric and spectrofluorimetric methods		
Estimation of sitagliptin alone		
Sr. No.	**Method**	**Reference**
1.	Validated UV Spectrophotometric Method for Estimation of Sitagliptin Phosphate in Tablet Dosage Form	[47]
2.	Development of First Order Derivative UV –Spectrophotometric Method for the Estimation of Sitagliptin Phosphate	[48]
3.	Development and validation of first order derivative UV-Spectrophotometric method for determination of Sitagliptin in bulk and in Formulation	[49]

4.	Development and validation of area under curve method by using first order derivative for estimation of Sitagliptin in tablet dosage form	[50]
5.	Analytical Method Development and Validation of Sitagliptine Phosphate Monohydrate in Pure and Tablet Dosage Form by UV-Vis Spectroscopy	[51]
6.	New Extractive Method Development of Sitagliptin Phosphate in API and Its Unit Dosage Forms by Spectrophotometry	[52]
7.	Spectrofluorimetric Method for Determination of Sitagliptin Phosphate in Formulation and Spiked Human Urine	[53]
8.	Analytical method development and validation of sitagliptine phosphate monohydrate in pure and tablet dosage form by derivative spectroscopy	[54]
9.	New extractive spectrophotometric estimation of sitagliptin phosphate and its dosage forms	[55]
	Simultaneous estimation of sitagliptin phosphate with other drugs	
1.	Development and validation of spectrophotometric method for estimation of Sitagliptin phosphate and Simvastatin in combined dosage form by derivative spectrophotometry	[56]
2.	Development and Validation of Spectrophotometric Method for Simultaneous Estimation of Sitagliptin Phosphate and Simvastatin in Tablet dosage form	[57]
3.	Simultaneous estimation of sitagliptin and pioglitazone by uv-spectroscopic method and study of interference of various excipients on this combination of drugs	[58]
4.	Simultaneous estimation of Sitagliptin and Metformin hydrochloride in Bulk and Dosage forms by UV-Spectrophotometry.	[59]
5.	Simultaneous UV Spectrophotometric Method for Estimation of Sitagliptin phosphate and Metformin hydrochloride in Bulk and Tablet Dosage Form	[60]
6.	Method Development Of Simultaneous Estimation Of Sitagliptin And Metformin Hydrochloride In Pure And Tablet Dosage Form By Uv-Vis Spectroscopy	[61]
7.	Spectrophotometric Determination Of Sitagliptin And Metformin In Their Pharmaceutical Formulation	[62]
8.	Simultaneous UV Spectrophotometric Method for Estimation of Sitagliptin Phosphate and Simvastatin in Tablet dosage Form.	[63]
9.	UV Spectroscopic Simultaneous Estimation Of Sitagliptin Phosphate And Metformin Hydrochloride In Bulk And In Dosage Form	[64]
10.	Development and validation of economic UV spectrophotometric method for simultaneous estimation of Sitagliptin phosphate and Simvastatin in bulk and tablet dosage form by absorption ratio method	[65]
11.	Development and Validation of Simultaneous Equation Method for the Estimation of Metformin and Sitagliptin by UV Spectroscopy	[66]
12.	New Validated Spectroscopic Method for the Simultaneous Estimation of Simvastatin and Sitagliptin	[67]
13.	Simultaneous estimation of simvastatin and sitagliptin by using different analytical methods	[68]

Chromatographic methods: Liquid chromatographic methods		
Estimation of sitagliptin alone		
Sr. No.	**Method**	**Reference**
1.	Development and Validation of RP-HPLC Method for the Estimation of Sitagliptin Phosphate in Tablet Dosage Form.	69
2.	Validation and Application of a High-Performance Liquid Chromatography Method for Estimation of Sitagliptin Phosphate from Bulk Drug and Pharmaceutical Formulation	70
3.	Bioanalytical Method Development And Validation Of Sitagliptin Phosphate By RP-HPLC And Its Application To Pharmacokinetic Study	71
4.	Simple, Rapid RP-HPLC Method For Estimation Of Sitagliptin From Urine And Its Application In Pharmacokinetics	72
5.	Stability-indicating RPHPLC method for analysis of sitagliptin in the bulk drug and its pharmaceutical dosage form	73
Simultaneous estimation of sitagliptin phosphate with other drugs		
Sr. No.	**Method**	**Reference**
1.	Simultaneous Estimation of Metformin HCl and Sitagliptin Phosphate in Tablet Dosage Forms by RP-HPLC	74
2.	Validated RP-HPLC method for simultaneous estimation of metformin hydrochloride and sitagliptin phosphate in bulk drug and pharmaceutical formulation	75
3.	RP-HPLC Method for the Simultaneous Estimation of Sitagliptin Phosphate and Metformin Hydrochloride in Combined Tablet Dosage Forms	76
4.	Development and validation of RP-HPLC method for simultaneous estimation of sitagliptin and simvastatin in bulk and tablet dosage forms	77
5.	RP-HPLC method for simultaneous determination of metformin hydrochloride, rosiglitazone and sitagliptin – application to commercially available drug products	78
6.	Simultaneous estimation of sitagliptin phosphate monohydrate and metformin hydrochloride in bulk and pharmaceutical formulation by RP-HPLC	79
7.	Method development and validation for simultaneous determination of sitagliptin phosphate and metformin hydrochloride by RP-HPLC in bulk and tablet dosage form	80
8.	Simultaneous Determination of Sitagliptin Phosphate Monohydrate and Metformin Hydrochloride in Tablets by a Validated UPLC Method	81
9.	Simultaneous estimation of sitagliptin and simvastatin in tablet dosage form by a validated RP-HPLC method	82
10.	Development of RP-HPLC method and it's validation for simultaneous estimation of sitagliptin and Metformin	83
11.	Development of stability indicating RP- HPLC method for simultaneous estimation of metformin hydrochloride and sitagliptin phosphate monohydrate in bulk as well as in pharmaceutical formulation	84
12.	Simultaneous Estimation of Sitagliptin Phosphate Monohydrate and Metformin Hydrochloride in Bulk and Pharmaceutical Formulation by RP-HPLC	85
13.	Development and Validation of a Stability Indicating RP-HPLC Method for Simultaneous Determination of Sitagliptin and Metformin in Tablet Dosage Form	86

14.	RP-HPLC method development and validation for simultaneous estimation of metformin and sitagliptin in tablet dosage forms	[87]
15.	A validated RP-HPLC method for simultaneous estimation of sitagliptin and simvastatin in tablet dosage form	[88]
16.	Development and validation of a new simple RP-HPLC method for estimation of Metformin HCl and Sitagliptin phosphate simultaneously in bulk and dosage forms	[89]
17.	Analytical method development and validation for the determination of sitagliptin and metformin using reverse phase HPLC	[90]
18.	A New Analytical Method Development and Validation for Simultaneous Estimation of Sitagliptin and Metformin Hydrochloride in Tablet Dosage form by RP-HPLC	[91]
19.	Validated RP-HPLC Method for the Estimation of Simvastatin and Sitagliptin	[92]
20.	A New, Simple, Sensitive, Accurate & Rapid Analytical Method Development & Validation for Simultaneous Estimation of Sitagliptin & Simvastatin in Tablet dosage form by using UPLC	[93]
21.	Development and Validation of RP-HPLC Method for Simultaneous Estimation of Sitagliptin and Simvastatin in Bulk and Tablet Dosage Form	[94]
22.	Stability indicating RP-HPLC method for simultaneous estimation of simvastatin and sitagliptin in tablet dosage form	[95]
Chromatographic methods: Thin layer chromatographic methods		
Sr. No.	**Method**	**Reference**
1.	A Simple and Sensitive HPTLC Method for Simultaneous Determination of Metformin Hydrochloride and Sitagliptin Phosphate in Tablet Dosage Form	[96]
2.	Validated HPTLC method for simultaneous estimation of metformin hydrochloride and sitagliptin phosphate in bulk drug and formulation	[97]
3.	Development and Validation of HPTLC method for the estimation of Sitagliptin Phosphate and Simvastatin in bulk and Marketed Formulation	[98]
4.	Validated HPTLC Method for the Simultaneous Determination of Metformin Hydrochloride and Sitagliptin Phosphate in Marketed Formulation	[99]
5.	Validated HPTLC Method for Simultaneous Estimation of Sitagliptin and Metformin Hydrochloride in Bulk Drug and Formulation	[100]
Other methods		
Sr. No.	**Method**	**Reference**
1.	Simultaneous Determination of Sitagliptin and Metformin in Pharmaceutical Preparations by Capillary Zone Electrophoresis and its Application to Human Plasma Analysis	[101]
2.	Development of UV-SPectrophotometry and RP-HPLC Method and Its Validation for Simultaneous Estimation of Sitagliptin Phosphate and Simvastatin in Marketed Formulation	[102]
3.	Development and Validation of RP-HPLC and HPTLC Methods for Simultaneous Estimation of Sitagliptin Phosphate and Metformin Hydrochloride in Bulk and Dosage form	[103]
4.	Chiral separation of sitagliptin phosphate enantiomer by HPLC using amylose based chiral stationary phase	[104]

3. AIM OF WORK

Stability is an essential factor for quality, safety and efficacy of a drug product. A drug product, which is not stable, can result in changes in physical (hardness, dissolution rate, phase separation etc.) as well as chemical characteristics (formation of high risk decomposition substances). Degradation study of drug itself and its pharmaceutical formulation allows a better knowledge of its therapeutic, physicochemical and toxicological behavior. The study of drug degradation kinetics is of greater importance for development of stable formulation and establishment of expiration date for commercially available drug products and also helps in deciding the routes of administration and storage conditions of various pharmaceutical dosage forms.

Literature describes degradation of repaglinide in acidic medium and degradation of sitagliptin in alkaline and acidic conditions. However, there was no published report found which describes the stability indicating HPTLC methods for estimation of ripaglinide and sitagliptin in their pharmaceutical dosage forms and degradation kinetics for both drugs.

Therefore, it was thought of interest to develop a simple, accurate, precise and specific stability indicating HPTLC methods for estimation of repaglinide and sitagliptin phosphate in pharmaceutical dosage forms and to extend their applicability to degradation kinetic study of respective drugs.

Hence the objectives of present work were

- To develop and validate stability indicating HPLC and HPTLC method for estimation of repaglinide in its pharmaceutical dosage forms
- Degradation kinetic study of repaglinide in acidic medium by HPTLC method
- Isolation and characterization of degradation products of repaglinide formed in acidic condition
- To develop and validate stability indicating HPLC and HPTLC method for estimation of sitagliptin phosphate in its pharmaceutical dosage forms
- Degradation kinetic study of sitagliptin phosphate in alkaline medium
- Isolation and characterization of degradation products of sitagliptin phosphate formed in alkaline condition

4. EXPERIMENTAL

4.1 INSTRUMENTS AND APPARATUS

Instruments

Electronic analytical Balance: Shimadzu AUX-220

Ultrasonicator: Janki Impex Pvt Ltd.

UV-Visible spectrophotometer: Shimadzu UV 1800 with two matched 1 cm quartz cells, UV probe 2.33 software

FT-IR spectrophotometer: Bruker Alpha-E (ATR module) with OPUS 6.5 software

HPTLC system: Camag Linomat V semiautomatic sample applicator with Hemilton syringe (100 µL), Camag twin trough developing chambers (10 x10 and 10x20), Camag TLC scanner IV, Camag winCATS software version1.4.6

HPLC system: Shimadzu liquid chromatograph LC-2010CHT with UV and PDA detector, Spincotech enable C18Q column (250 x 4.6 mm, 5 µm), automatic sample injection and column temperature controller

Digital pH meter: Elico Ltd.

Controlled temperature water bath: Janki Impex Pvt. Ltd.

Hot air oven:

Apparatus

All the apparatus used are made of borosilicate glass type I.

Apparatus	Nominal capacity (mL)
Volumetric flask	10, 25, 50, 100, 250, 1000
One-mark pipettes	5, 10, 25
Graduated pipettes	1, 2, 5,10
Measuring cylinder	10, 50
Beakers	100, 250, 500
Thiele's tube	NA

4.2 REAGENTS AND MATERIALS

Repaglinide (Gift sample from Torrent Pharma, Ahmedabad); Sitagliptin; Whatman filter paper No. 41; Pre-coated silica plates; Toluene AR grade; Methanol Extra pure; Chloroform LR grade; Acetonitrile HPLC grade; Double distilled water; Ammonia solution; Concentrated Hydrochloric acid; Glacial acetic acid; Ortho phosphoric acid AR grade; Sodium hydroxide pellets; Hydrogen peroxide solution

4.3 METHOD DEVELOPMENT AND VALIDATION FOR REPAGLINIDE

4.3.1 HPTLC METHOD

Standard solutions of repaglinide were prepared in methanol: Stock standard solution of repaglinide (1 mg/mL), Working standard solution A (100 µg/mL) and Working standard solution B (40 µg/mL). Repaglinide was degraded in acidic (1N HCl at 80° C for 6 hrs), alkaline (1 N NaOH at 80° C for 6 hrs), oxidative (3% hydrogen peroxide at room temperature for 24 hrs), photolytic (direct sunlight for 6 hrs) and thermal (at 120° C for 3 hrs) stress conditions. Degraded solutions in acid and alkali were neutralized to pH 7. These solutions were utilized for mobile phase optimization to get separation of drug from degradation products.

The stock standard solution of repaglinide and degraded drug solutions were spotted separately on pre-coated silica gel aluminium plates by using glass capillary tube and allowed to dry in hot air oven. The spotted plates were developed in different mobile phases about ¾ height of the plate. The plates were removed and allowed them to dry in hot air oven. Spots were observed in U.V cabinet for tailing, shape, separation etc. Once proportion of different solvents in mobile phase was finalized, saturation time for mobile phase was also optimized. Wavelength of detection was selected from overlain UV spectra of repaglinide and degradation products.

Separation was performed using optimized chromatographic conditions as follow.

- ⇒ Stationary Phase- Aluminum plates precoated with silica gel 60 F_{254} (10 x 10, cm)
- ⇒ Mobile Phase- Toluene: Acetonitrile: Glacial acetic acid (5: 5: 0.1, v/v/v)
- ⇒ Chamber saturation time- 20 min
- ⇒ Migration distance- 80 mm
- ⇒ Application parameters
  - Syringe: 100 µL
  - Application rate: 100 nL/s
  - Band width: 6 mm
  - Distance from plate edge: 15 mm
  - Distance from bottom of the plate: 15 mm
- ⇒ Scanning parameters
  - Slit dimension: 4 mm x 0.20 mm
  - Scanning speed: 20 mm/s
  - Detection wavelength: 245 nm
  - Lamp: D2
  - Measurement mode: Absorption/Reflectance

Calibration curve was prepared by spotting appropriate volume of working standard solution on a TLC plate to obtain concentration in range of 200-1000 ng/spot. Calibration curve was constructed by plotting peak area of repaglinide versus respective concentrations. The developed method was validated for specificity, linearity, precision, accuracy, LOD and LOQ as per ICH guidelines.

Twenty tablets were individually weighed and powdered. Tablet powder equivalent to 2 mg of repaglinide was accurately weighted, transferred to 10 mL volumetric flask and 7 mL of methanol was added. Solution was sonicated for 15 minutes and volume was made up to mark with methanol. The resulting solution was filtered through Whatman filter paper no. 41. 2 mL filtrate was diluted to 10 mL with methanol. Resulting solution (15 µL) applied in triplicate on TLC plate followed by development and scanning as optimized chromatographic conditions.

All degraded solutions were appropriately diluted, resulting solutions (20 µL) were applied to TLC plate chromatogram was developed as described above. Area of peak corresponding to repaglinide was measured and percent of drug degraded was calculated from calibration curve.

4.3.2 HPLC METHOD

Standard solutions of repaglinide were prepared in mixture of acetonitrile and water (65:35 v/v): Stock standard solution of repaglinide (1 mg/mL), Working standard solution A (100 µg/mL) and Working standard solution B (40 µg/mL). Repaglinide was degraded in acidic (1N HCl at 80° C for 6 hrs), alkaline (1 N NaOH at 80° C for 6 hrs), oxidative (3% hydrogen peroxide at room temperature for 24 hrs), photolytic (direct sunlight for 6 hrs) and thermal (at 120° C for 3 hrs) stress conditions. Degraded solutions in acid and alkali were neutralized to pH 7. These solutions were utilized for mobile phase optimization to get separation of drug from degradation products.

Initial trials were conducted to select a suitable solvent system for accurate estimation of drug and to achieve good resolution between the drug and degradation products. Various proportions of methanol, acetonitrile and water were tried on the basis of sensitivity of assay, suitability for stability studies, time required for analysis, ease of preparation and use of readily available cost effective solvents. Chromatograms were evaluated for better separation and peak shapes. Wavelength of detection was selected from overlain UV spectra of repaglinide standard and degradation solutions. Separation was performed using optimized chromatographic conditions as follow.

- Stationary Phase- C_{18} (Grace Smart RP 18, 5µm, 250mm x 4.6mm)
- Mobile Phase- Acetonitrile : Water (pH adjusted to 3.0 with ortho phosphoric acid) [65:35 v/v]
- Flow Rate- 1.0 mL/min
- Detector- UV
- Detection wavelength- 245 nm
- Temperature- 25 ± 2°C
- Total run time- 10 minutes

System suitability tests are an integral part of liquid chromatography. The resolution, column efficiency and peak symmetry were calculated for the standard solution and degraded solutions and compared with USP specifications.

Calibration curve was prepared by injecting appropriate volume of working standard solution to obtain concentration in range of 200-1000 ng/spot. Calibration curve was constructed by plotting peak area of repaglinide versus respective concentrations. The developed method was validated for specificity, linearity, precision, accuracy, LOD and LOQ as per ICH guidelines.

Twenty tablets were individually weighed and powdered. Tablet powder equivalent to 2 mg of repaglinide was accurately weighted, transferred to 10 mL volumetric flask and 7 mL of methanol was added. Solution was sonicated for 15 minutes and volume was made up to mark with methanol. The resulting solution was filtered through Whatman filter paper no. 41. Two mL filtrate was diluted to 10 mL with methanol. Resulting solution (15 µL) was injected to HPLC system in triplicate and analysed as described above.

All degraded solutions were appropriately diluted, resulting solutions (20 µL) were injected to HPLC system and analysed. Area of peak corresponding to repaglinide was measured and percent of drug degraded was calculated from calibration curve.

4.4 METHOD DEVELOPMENT AND VALIDATION FOR SITAGLIPTIN PHOSPHATE

4.4.1 HPTLC METHOD

Standard solutions were prepared in double distilled water: Stock standard solution of sitagliptin phosphate (1 mg/mL), Working standard solution (100 µg/mL). Sitagliptin was degraded in acidic (1N HCl at 80° C for 4 hrs), alkaline (0.1 N NaOH at 80° C for 4 hrs), oxidative (3% hydrogen peroxide at room temperature for 24 hrs), photolytic (direct sunlight for 4 hrs) and thermal (at 120° C for 3 hrs) stress conditions. Degraded solutions in acid and alkali were neutralized to pH 7. These solutions were utilized for mobile phase optimization to get separation of drug from degradation products.

The stock standard solution of sitagliptin phosphate and degraded drug solutions were spotted separately on pre-coated silica gel aluminium plates by using glass capillary tube and allowed to dry in hot air oven. The spotted plates were developed in different mobile phases about ¾ height of the plate. The plates were removed and allowed them to dry in hot air oven. Spots were observed in U.V cabinet for tailing, shape, separation etc. Once proportion of different solvents in mobile phase was finalized, saturation time for mobile phase was also optimized. Wavelength for detection was selected from overlay UV spectra of working standard solution of sitagliptin phosphate and degradation solutions. Separation was performed using optimized chromatographic conditions as follow.

- ⇒ Stationary Phase- Aluminum plates precoated with silica gel 60 F_{254} (10 x 10, cm)
- ⇒ Mobile Phase- Chloroform: methanol: triethyamine (8: 2: 0.2, v/v/v)
- ⇒ Chamber saturation time- 20 min
- ⇒ Migration distance- 70 mm
- ⇒ Application parameters
  - Syringe: 100 µL
  - Application rate: 100 nL/s
  - Band width: 6 mm
  - Distance from plate edge: 15 mm
  - Distance from bottom of the plate: 15 mm
- ⇒ Scanning parameters
  - Slit dimension: 4 mm x 0.20 mm
  - Scanning speed: 20 mm/s
  - Detection wavelength: 267 nm
  - Lamp: D2
  - Measurement mode: Absorption/Reflectance

Calibration curve was prepared by spotting appropriate volume of working standard solution on a TLC plate to obtain concentration in range of 500-25000 ng/spot. Calibration curve was constructed by plotting peak area of sitagliptin phosphate versus respective concentrations. The developed method was validated for specificity, linearity, precision, accuracy, LOD and LOQ as per ICH guidelines.

Twenty tablets were individually weighed and powdered. Tablet powder equivalent to 10 mg of sitagliptin phosphate was accurately weighted, transferred to 100 mL volumetric flask and 50 mL of double distilled water was added. Solution was sonicated for 15 minutes and volume was made up to mark with double distilled water. The resulting solution was filtered through Whatman filter paper no. 41. Filtrate (15 µL) was applied in triplicate on TLC plate followed by development and scanning as described above.

All degraded solutions were appropriately diluted, resulting solutions (8.5 µL) were applied to TLC plate and chromatogram was developed. Area of peak corresponding to sitagliptin phosphate was measured and percent of drug degraded was calculated from calibration curve.

4.4.2 HPLC METHOD

Standard solutions of sitagliptin phosphate were prepared in mixture of acetonitrile and double distilled water (65:35 v/v): Stock standard solution (1 mg/mL), working standard solution (100µg/mL).

Sitagliptin was degraded in acidic (1N HCl at 80° C for 4 hrs), alkaline (0.1 N NaOH at 80° C for 4 hrs), oxidative (3% hydrogen peroxide at room temperature for 24 hrs), photolytic (direct sunlight for 4 hrs) and thermal (at 120° C for 3 hrs) stress conditions. Degraded solutions in acid and alkali were neutralized to pH 7. These solutions were utilized for mobile phase optimization to get separation of drug from degradation products.

Initial trials were conducted to select a suitable solvent system for accurate estimation of drug and to achieve good resolution between the drug and degradation products. Various proportions of methanol, acetonitrile and water were tries on the basis of sensitivity of assay, suitability for stability studies, time required for analysis, ease of preparation and use of readily available cost effective solvents. Chromatograms were evaluated for better separation and peak shapes. Wavelength for detection was selected from overlay UV spectra of working standard solution of sitagliptin phosphate and degradation solutions. Separation was performed using optimized chromatographic conditions as follow.

- Stationary Phase- C18 (Grace Smart RP 18, 5µm, 250mm x 4.6mm)
- Mobile Phase- Acetonitrile : Water (0.5% triethylamine; pH adjusted to 6.8 with ortho phosphoric acid) [65:35 v/v]
- Flow Rate- 1.0 mL/min
- Detector- UV
- Detection wavelength- 267 nm
- Temperature- 25 ± 2°C
- Total run time- 10 minutes

System suitability tests are an integral part of liquid chromatography. The resolution, column efficiency and peak symmetry were calculated for the standard solution and degraded solutions and compared with USP specifications.

Calibration curve was prepared by injecting appropriate volume of working standard solution to obtain concentration in range of 500-2500 ng/spot. Calibration curve was constructed by plotting peak area of sitagliptin phosphate versus respective concentrations. The developed method was validated for specificity, linearity, precision, accuracy, LOD and LOQ as per ICH guidelines.

Twenty tablets were individually weighed and powdered. Tablet powder equivalent to 10 mg of sitagliptin phosphate was accurately weighted, transferred to 100 mL volumetric flask and 50 mL of double distilled water was added. Solution was sonicated for 15 minutes and volume was made up to mark with double distilled water. The resulting solution was filtered through Whatman filter paper no. 41. Filtrate (15 µL) was injected to HPLC system in triplicate and analysed as described above.

All degraded solutions were appropriately diluted, resulting solutions (15 µL) injected in HPLC system and chromatograms were recorded as described above. Area of peak corresponding to sitagliptin phosphate was measured and percent of drug degraded was calculated from calibration curve.

4.5 ISOLATION AND CHARACTERIZATION OF DEGRADATION PRODUCTS

4.5.1 REPAGLINIDE

Accurately weighted 500mg of repaglinide was completely degraded in hydrochloric acid solution. The degradation products were separated by extraction of completely degraded solution. The Mass spectrum, UV spectrum, NMR spectrum, IR spectrum and melting point of degradation products were recorded for identification and characterization of degradation products.

4.5.2 SITAGLIPTIN PHOSPHATE

Accurately weighted 500mg of sitagliptin phosphate was completely degraded in sodium hydroxide solution. The degradation products were separated by extraction of completely degraded solution. The Mass spectrum, UV spectrum, NMR spectrum, IR spectrum and melting point of degradation products were recorded for identification and characterization of degradation products.

4.6 DEGRADATION KINETIC STUDY OF SITAGLIPTIN PHOSPHATE IN ALKALINE MEDIUM

Degradation kinetics was studied for sitagliptin phosphate in alkaline medium at three different alkali concentrations (0.1 M, 0.5 M and 1 M NaOH) and three different temperatures (25 ± 2°C, 50 ± 2°C and 80 ± 2°C). Area of drug peak was measured. The concentration of drug remained unchanged was determined from the regression line equation and % degradation of drug was calculated.

5. RESULTS AND DISCUSSION

5.1 METHOD DEVELOPMENT AND VALIDATION FOR REPAGLINIDE

5.1.1 HPTLC METHOD

From all various compositions of different solvents tried as mobile phase, the mixture of Toluene: acetonitrile: acetic acid (7:3:0.1 v/v/v) was found to give a separate and resolved spot of repaglinide (R_f=0.60) from its degradation products with better peak shapes. UV spectra of standard solution of repaglinide as well as of degradation solutions have shown good absorbance at 245 nm. Therefore 245 nm was selected as a wavelength of detection. Calibration curve was prepared in range of 200-1000 ng/spot. Peak area of repaglinide was found to be linear in this range.

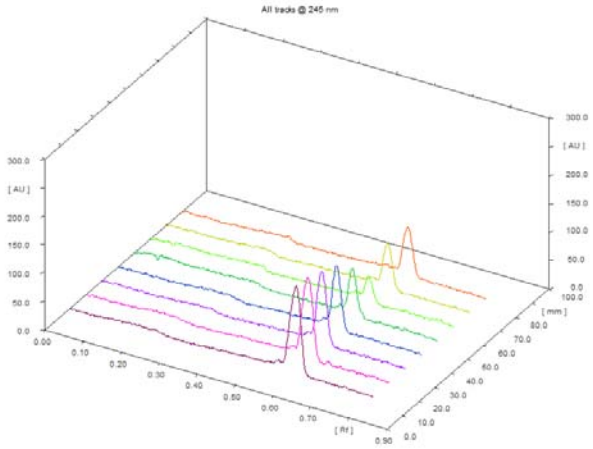


Figure 1- 3D chromatogram of calibration levels of repaglinide (200-1000 ng/spot)

Linearity of responses were found be in a range of 200-1000 ng/spot. The RSD of repeatability, intra-day precision and inter-day precision were found to be less than 2 % for repaglinide at selected wavelength. The % recovery at each level was found to be within range of 98 to 102% for repaglinide at selected wavelength.

The proposed method was applied for assay of tablets containing repaglinide and the assay values were found to be 99.69 ± 1.81 % of labeled claim of repaglinide.

Repaglinide was found to be degraded in acidic and oxidative stress conditions with two additional peaks present in chromatogram of acid degraded solutions, while one additional peak in hydrogen peroxide degraded solutions as compared to chromatogram of standard repaglinide. Repaglinide was found to be stable in photolytic and alkaline stress conditions with no additional peak present in chromatograms.

5.1.2 HPLC METHOD

From various compositions of methanol, acetonitrile and water tried as mobile phase, the mobile phase system of acetonitrile : water (pH adjusted to 3.0 with orthophosphoric acid) (65:35 v/v) at a flow rate of 1 mL/min with UV detection at 245 nm was found to give a separate and resolved peak of Sitagliptin (R_t= 5.2 min) from its degradation products with better peak shapes. UV spectra of standard solution of repaglinide as well as of degradation solutions have shown good absorbance at 245 nm. Therefore 245 nm was selected as a wavelength of detection.

System suitability parameters like Resolution, Number of theoretical plates, tailing factor were found to comply with USP specifications.

Calibration curve was prepared in range of 200-1000 ng/injection. The results show excellent correlation (r^2= 0.9993) between peak area and concentration.

Linearity of detector responses were found be in a range of 200-1000 ng/injection. The RSD of repeatability, intra-day precision and inter-day precision were found to be less than 2 % for sitagliptin phosphate at selected wavelength. The % recovery at each level was found to be within range of 98 to 102% for sitagliptin phosphate at selected wavelength.

The proposed method was applied for assay of tablets containing sitagliptin phosphate and the assay values were found to be 99.02 ± 1.12 % of labeled claim of sitagliptin phosphate.

Repaglinide was found to be degraded in acidic and oxidative stress conditions with two additional peaks present in chromatogram of acid degraded solutions, while one additional peak in hydrogen peroxide degraded solutions as compared to chromatogram of standard repaglinide. Repaglinide was found to be stable in photolytic and alkaline stress conditions with no additional peak present in chromatograms.

5.2 METHOD DEVELOPMENT AND VALIDATION FOR SITAGLIPTIN PHOSPHATE

5.2.1 HPTLC METHOD

From all various compositions of different solvents tried as mobile phase, the mixture of chloroform : methanol : triethylamine (8:2:0.2 v/v/v) was found to give a separate and resolved spot of sitagliptin phosphate (R_f=0.42) from its degradation products with better peak shapes. The overlay spectra of degradation solutions have also shown absorbance at the wavelength maximum (267 nm) of sitagliptin phosphate. Therefore 267 nm was selected as a wavelength of detection.

Calibration curve was prepared in range of 500-2500 ng/spot. Calibration curve of sitagliptin 3D chromatogram of calibration levels are presented below.

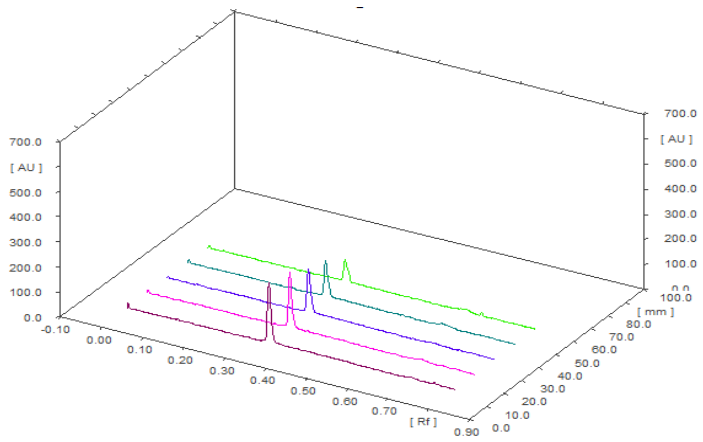


Figure 2- 3D chromatogram of calibration levels of sitagliptin phosphate (500-2500 ng/spot)

Linearity of responses were found be in a range of 500-2500 ng/spot. The RSD of repeatability, intra-day precision and inter-day precision were found to be less than 2 % for sitagliptin phosphate at selected wavelength. The % recovery at each level was found to be within range of 98 to 102% for sitagliptin phosphate at selected wavelength.

The proposed method was applied for assay of tablets containing sitagliptin phosphate and the assay values were found to be 98.55 ± 1.1 % of labeled claim of sitagliptin phosphate.

Sitagliptin phosphate was found to be degraded in acidic, alkaline and oxidative stress conditions with two additional peaks present in chromatogram of alkaline degraded solutions, while one additional peak in acidic and hydrogen peroxide degraded solutions as compared to chromatogram of standard sitagliptin phosphate. Sitagliptin phosphate was found to be stable in photolytic and thermal (dry heat) stress conditions with no additional peak present in chromatograms.

5.2.2 HPLC METHOD

From various compositions of methanol, acetonitrile and water tried as mobile phase, the mobile phase system of acetonitrile : water (0.5 % triethyleamine, pH adjusted to 6.8 with orthophosphoric acid) (65:35 v/v) at a flow rate of 1 mL/min with UV detection at 267 nm was found to give a separate and resolved peak of sitagliptin phosphate (Rt=3.2 min) from its degradation products with better peak shapes. The overlay spectra of degradation solutions have also shown absorbance at the wavelength maximum (267 nm) of sitagliptin phosphate. Therefore 267 nm was selected as a wavelength of detection. System suitability parameters like Resolution, Number of theoretical plates, tailing factor were found to comply with USP specifications. Calibration curve was prepared in range of 500-2500 ng/injection. The results show excellent correlation (r^2= 0.9986) between peak area and concentration.

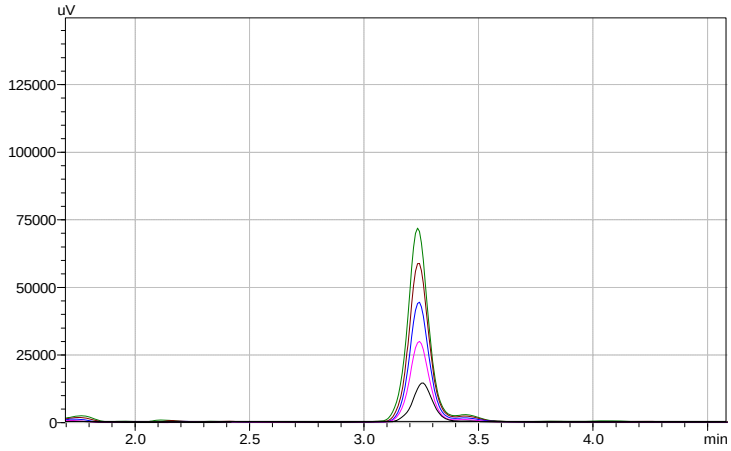


Figure 3- Overlay chromatograms of calibration levels of sitagliptin phosphate (500-2500 ng/injection)

Linearity of detector responses were found be in a range of 500-2500 ng/injection. The RSD of repeatability, intra-day precision and inter-day precision were found to be less than 2 % for sitagliptin phosphate at selected wavelength. The % recovery at each level was found to be within range of 98 to 102% for sitagliptin phosphate at selected wavelength.

The proposed method was applied for assay of tablets containing sitagliptin phosphate and the assay values were found to be 99.18 ± 0.68 % of labeled claim of sitagliptin phosphate.

Sitagliptin phosphate was found to be degraded in acidic, alkaline and oxidative stress conditions with two additional peaks present in chromatogram of alkaline degraded solutions, while one additional peak in acidic and hydrogen peroxide degraded solutions as compared to chromatogram of standard sitagliptin phosphate. Sitagliptin phosphate was found to be stable in photolytic and thermal (dry heat) stress conditions with no additional peak present in chromatograms.

5.3 ISOLATION AND CHARACTERIZATION OF DEGRADATION PRODUCTS

5.3.1 REPAGLINIDE

Repaglinide standard was spotted on TLC plate with its acidic degradation products and developed in Toluene: acetonitrile: acetic acid (7:3:0.1 v/v/v) in previously saturated TLC chamber. The TLC plate was observed under UV light in UV cabinet. The spots of degradation product 1 (DP1) and degradation product 2 (DP2) were found to be separated from the spot of repaglinide. Further characterization of degradation products was performed using IR, Mass and NMR spectra.

5.3.2 SITAGLIPTIN PHOSPHATE

Sitagliptin standard was spotted on TLC plate with its alkaline degradation product and developed in Chloroform: methanol: triethyamine (8: 2: 0.2, v/v/v) in previously saturated TLC chamber. The TLC plate was observed under UV light in UV cabinet. The spot of degradation product was found to be separated form spot of Sitagliptin phosphate. Further characterization of degradation products was performed using IR, Mass and NMR spectra.

5.4 DEGRADATION KINETICS STUDY OF SITAGLIPTIN PHOSPHATE IN ALKALINE MEDIUM

Alkaline degradation of sitagliptin phosphate was found to follow first order kinetics except in 1 M NaOH at 80° C where it seems to follow second order kinetics. Rate constant, half life and shelf life were calculated according to respective order. The degradation rate constant and half-life for alkaline degradation of sitagliptin phosphate were found to be highest in 1.0 M NaOH at 80° C temperature.

6. CONCLUSION

Simple, specific, precise and accurate stability indicating HPLC and HPTLC methods for estimation of repaglinide and sitagliptin phosphate have been developed and validated as per ICH guideline. Proposed methods have been applied for estimation of both respective drugs in their dosage forms. The developed HPTLC method for sitagliptin phosphate was extended for the degradation kinetic study of sitagliptin phosphate in alkaline conditions. Alkaline degradation of sitagliptin phosphate was found to follow first order kinetics at low temperature while it follows second order kinetics at high temperature. Degradation rate of sitagliptin phosphate increases as either strength of medium or temperature or both increases. Structures of degradation products were elucidated using IR, NMR and Mass spectra.

7. REFERENCES

1. Hardman, J. G., Limbird, L.E., Gilman, A. G. *Goodman & Gilman's The Pharmacological Basis of Therapeutics: Tenth edition*. 1679-1714 (McGraw Hill Professional, 2001).

2. Rang, H. P., Dale, M. M., Ritter, J. M., Flower, R. J. & Henderson, G. *Rang & Dale's Pharmacology: Sixth edition.* 397-409 (Elsevier Health Sciences, 2011).

3. Lemke, T. L., Williams D. A. *Foye's Principles of Medicinal Chemistry: Seventh edition*. 877-906 (Lippincott Williams & Wilkins, 2012).

4. Trevor, A., Katzung, B. & Masters, S. *Katzung & Trevor's Pharmacology Examination and Board Review: Eighth Edition*. 631 (McGraw Hill Professional, 2008).

5. Kenkre, J., Tan, T. & Bloom, S. Treating the Obese Diabetic. *Expert Rev. Clin. Pharmacol.* **6,** 171–183 (2003).

6. McDougall, C., McKay, G. A. & Fisher, M. Drugs for diabetes: part 5 DPP-4 inhibitors. *Br J Cardiol* **18,** 130–132 (2011).

7. Carstensen, J. T., Rhodes, C. T.*Drug Stability, Principles and Practices:Third Edition*. 19-133 (Taylor & Francis, 2000).

8. Moffat, A. C., Osselton, M. D., Widdop, B. *Clarke's Analysis of Drugs and Poisons: Third edition*. **2**, 1592 (Pharmaceutical Press, 2011).

9. O'neil, M. J.*The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals: Fourteenth edition*. 1402, 1474 (Merck Reserch Laboratory, 2006).

10. Pharmacopoeia commission, Department of Health. *British Pharmacopoeia*. **3,** 1878-1880 (Stationery Office, 2007).

11. The U.S. Pharmacopeial convention. *United states Pharmacopoeia 30- National Formulary 25*. **2**, 294 (USP Convention, 2007).

12. Sweetman, S. C. *Martindale: The Complete Drug Reference. Part 1: Thirty fifth edition*. 415, 417 (Pharmaceutical Press, 2011).

13. Block, J. H., Beale, J. M. *Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry: Eleventh edition*. 668-673 (Lippincott Williams & Wilkins, 2011).

14. Sitagliptin phosphate. at <http://pubchem.ncbi.nlm.nih.gov/compound/46782923#section=Top>

15. Singhvi, I. & Goyal, A. Visible spectrophotometric methods for estimation of repaglinide in tablet formulation. *Indian J. Pharm. Sci.* **68,** 656 (2006).

16. Kaushal, N., Jain, S. & Tiwary, A. K. Development of Spectrofluorimetric and HPLC Methods for In vitro Analysis of Repaglinide. *Indian J. Pharm. Sci.* **72,** 240–244 (2010).

17. Farouk, M., Abdel-Satar, O., Abdel-Aziz, O. & Shaaban, M. Validated spectrophotometric methods for determination of some oral hypoglycemic drugs. *Drug Discov. Ther.* **5,** 41–52 (2011).

18. Dhole, S. M., Khedekar, P. B. & Amnerkar, N. D. Comparison of UV spectrophotometry and high performance liquid chromatography methods for the determination of repaglinide in tablets. *Pharm. Methods* **3,** 68–72 (2012).

19. El-Shaheny, R. N. Validated stability-indicating spectrofluorimetric method with enhanced sensitivity for determination of repaglinide in tablets. *J. Fluoresc.* **22,** 1587–1594 (2012).

20. AlKhalidi, B. A., Shtaiwi, M., AlKhatib, H. S., Mohammad, M. & Bustanji, Y. A comparative study of first-derivative spectrophotometry and column high-performance liquid chromatography applied to the determination of repaglinide in tablets and for dissolution testing. *J. AOAC Int.* **91,** 530–535 (2008).

21. Sankar, D. & Pawar, A. Spectrophotometric determination of repaglinide and ezetimibe. *Asian J.Chem.* **17** (3), 2025-2027 (2005).

22. Patel, J., Suhagia, B. & Patel, B. Simultaneous spectrophotometric estimation of metformin and repaglinide in a synthetic mixture. *Indian J. Pharm. Sci.* **69,** 844 (2007).

23. Prasanth, V. & Eapen, S. Development and validation of UV spectroscopic methods for the estimation of Repaglinide and Metformin hydrochloride in synthetic mixture. *Int J Pharm Sci Heal.* **2** (2), 150-158 (2012).

24. Greischel, A., Beschke, K., Rapp, H. & Roth, W. Quantitation of the new hypoglycaemic agent AG-EE 388 ZW in human plasma by automated high-performance liquid chromatography with electrochemical detection. *J. Chromatogr.* **568,** 246–252 (1991).

25. Gandhimathi, M., Ravi, T. K. & Renu, S. K. Determination of repaglinide in pharmaceutical formulations by HPLC with UV detection. *Anal. Sci.* **19,** 1675–1677 (2003).

26. Ruzilawati, A. B., Wahab, M. S. A., Imran, A., Ismail, Z. & Gan, S. H. Method development and validation of repaglinide in human plasma by HPLC and its application in pharmacokinetic studies. *J. Pharm. Biomed. Anal.* **43,** 1831–1835 (2007).

27. Prameela, R., Bala, S., Archana, N., Siva, T. & Aruna, B. Determination of repaglinide in pharmaceutical formulation by HPLC. *J Appl Sci Res*. **5**, 1500-1504 (2009).

28. Sharma, M. & Sharma, S. Stability Indicating RP-HPLC Method for Determination and Validation of Repaglinide in Pharmaceutical Dosage Form. *Int. J. ChemTech.* **3**(1), 210-216 (2011).

29. Ho, E. N. M., Yiu, K. C. H., Wan, T. S. M., Stewart, B. D. & Watkins, K. L. Detection of anti-diabetics in equine plasma and urine by liquid chromatography-tandem mass spectrometry. *J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci.* **811,** 65–73 (2004).

30. Berecka, A., Gumieniczek, A. & Hopkała, H. Development and validation of a new high-performance liquid chromatography method for the determination of gliclazide and repaglinide in pharmaceutical formulations. *J. AOAC Int.* **89,** 319–325 (2006)

31. Venkatesh, P., Harisudhan, T., Choudhury, H., Mullangi, R. & Srinivas, N. R. Simultaneous estimation of six anti-diabetic drugs--glibenclamide, gliclazide, glipizide, pioglitazone, repaglinide and rosiglitazone: development of a novel HPLC method for use in the analysis of pharmaceutical formulations and its application to human plasma. *Biomed. Chromatogr.* **20,** 1043–1048 (2006).

32. Wang, M. & Miksa, I. R. Multi-component plasma quantitation of anti-hyperglycemic pharmaceutical compounds using liquid chromatography-tandem mass spectrometry. *J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci.* **856,** 318–327 (2007).

33. Yao, J., Shi, Y.-Q., Li, Z.-R. & Jin, S.-H. Development of a RP-HPLC method for screening potentially counterfeit anti-diabetic drugs. *J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci.* **853,** 254–259 (2007).

34. Petsalo, A., Turpeinen, M., Pelkonen, O. & Tolonen, A. Analysis of nine drugs and their cytochrome P450-specific probe metabolites from urine by liquid chromatography-tandem mass spectrometry utilizing sub 2 microm particle size column. *J. Chromatogr. A* **1215,** 107–115 (2008).

35. Hess, C., Musshoff, F. & Madea, B. Simultaneous identification and validated quantification of 11 oral hypoglycaemic drugs in plasma by electrospray ionisation liquid chromatography-mass spectrometry. *Anal. Bioanal. Chem.* **400,** 33–41 (2011).

36. Lakshmi, K. S. & Rajesh, T. Separation and quantification of eight antidiabetic drugs on a high-performance liquid chromatography: its application to human plasma assay. *ISRN Pharm.* **2011,** 521-353 (2011).

37. Soni, L. K., Narsinghani, T. & Jain, M. Development And Validation Of Rp-Hplc Method For Simultaneous Estimation Of Metformin Hydrochloride And Repaglinide In Tablet Dosage Form. *J. Liq. Chromatogr. Relat. Technol.* **35,** 385–392 (2012).

38. Liang, X.-R. *et al.* Liquid chromatography-tandem mass spectrometry simultaneous determination of repaglinide and metformin in human plasma and its application to bioequivalence study. *Yao Xue Xue Bao* **48,** 547–553 (2013).

39. Sharma, K. *et al.* LC-MS/MS-ESI method for simultaneous quantitation of metformin and repaglinidie in rat plasma and its application to pharmacokinetic study in rats. *Biomed. Chromatogr.* **27,** 356–364 (2013).

40. Gumieniczek, A. Quantitative analysis of repaglinide in tablets by reversed-phase thin-layer chromatography with densitometric UV detection. *J Plan Chromatogra.* **18** (102), 155-159 (2005).

41. Meeta, A. & Saurabh, S. Estimation of Repaglinide in bulk and tablet dosage forms by HPTLC method. *Int. J. Pharm Pharm Sci.* **1** (1), 21 (2009).

42. Rane, V. & Shinde, D. A validated chiral LC method for the enantiomeric separation of repaglinide on amylose based stationary phase. *Chromatographia*. **66** (7-8), 583-587 (2007).

43. El-Ries, M., Mohamed, G. & Attia, A. Electrochemical determination of the antidiabetic drug repaglinide. *Yakugaku Zasshi* **128** (1), 171-177 (2008).

44. Maier, V. *et al.* Determination of antihyperglycemic drugs in nanomolar concentration levels by micellar electrokinetic chromatography with non-ionic surfactant. *J. Chromatogr. A* **1216,** 4492–4498 (2009).

45. Jirovsky, D., Bartosova, Z., Skopalova, J. & Maier, V. Electrochemical characterization of repaglinide and its determination in human plasma using liquid chromatography with dual-channel coulometric detection. *J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci.* **878,** 3243–3248 (2010).

46. Li, C. & Jiang, Y. Analysis of repaglinide enantiomers in pharmaceutical formulations by capillary electrophoresis using 2,6-di-o-methyl-β-cyclodextrin as a chiral selector. *J. Chromatogr. Sci.* **50,** 739–743 (2012).

47. Patil, S. & Ramesh, B. Validated UV Spectrophotometric Method for Estimation of Sitagliptin Phosphate in Tablet Dosage Form. *Res. J.* **3** (3), 798-800 (2010).

48. Ghodeswar, P., Jain, A. & Jain, S. Development of First Order Derivative UV–Spectrophotometric Method for the Estimation of Sitagliptin Phosphate. *Res. J. Pharm.* **4** (4), 631-633 (2011).

49. Pritam, J., Amar, C., Bhargav, D. & Shani, P. Development and validation of first order derivative UV-Spectrophotometric method for determination of Sitagliptin in bulk and in formulation. *Int J Drug Dev* **3,** 194–199 (2011).

50. Jain, P., Chaudhari, A., Patil, A. & Surana, S. Development and validation of area under curve method by using first order derivative for estimation of Sitagliptin in tablet dosage form. *Anal chem* **11** (9,10) (2012).

51. Jeyabalan, G. & Nyola, N. Analytical Method Development and Validation of Sitagliptine Phosphate Monohydrate in Pure and Tablet Dosage Form by UV-Vis Spectroscopy. *Res. Rev. J.* **1**(1), 19-23 (2012).

52. Lavanya, G., Prakash, K., Kumar, Y. & Moin, A. New Extractive Spectrophotometric Estimation Of Sitagliptin Phosphate And Its Dosage Forms. *Int. Res J Pharm. App Sci.* **3** (4), 19-21 (2013)

53. Havelikar, H., Soni, N. & Shah, P. Spectrofluorimetric Method for Determination of Sitagliptin Phosphate in Formulation and Spiked Human Urine. *Int J Anal and Bioanal Chem.* **2**(4), 214-217 (2012).

54. Jeyabalan, G. & Nyola, N. Analytical method development and validation of sitagliptine phosphate monohydrate in pure and tablet dosage form by derivative spectroscopy. *World J Pharma and Pharm Sci* **1**(4) 1392-1401 (2013).

55. Monila, N., Pulla, R. & Shabad, H. New extractive method development of sitagliptin phosphate in API and its unit dosage forms by spectrophotometry. *IOSR J Pharm Biol Sci* **1**(6), 37-40 (2012).

56. Abirami, G. & Vetrichelvan, T. Development and validation of spectrophotometric method for estimation of Sitagliptin phosphate and Simvastatin in combined dosage form by derivative spectrophotometry. *J. Pharm. Res.* **5** (12), 5549 (2012).

57. Kothari, A. & Sharma, S. Development And Validation Of Spectrophotometric Method For Simultaneous Estimation Of Sitagliptin Phosphate And Simvastatin In Tablet Dosage Form. *Inter J Pharm* **5** (1), 444-446 (2012).

58. Kumar, S., Krishnaveni, Y. & Ramesh, G. Simultaneous estimation of sitagliptin and pioglitazone by UV-spectroscopic method and study of interference of various excipients on this combination of drugs. *Int J Curr Pharm Res* **4,** 267–270 (2012).

59. Kupkar, S. Simultaneous estimation of Sitagliptin and Metformin hydrochloride in Bulk and Dosage forms by UV-Spectrophotometry. *Int. J. Pharm. Res.* **5**(1), 580-582**.** (2012).

60. Loni, A. Simultaneous UV Spectrophotometric Method for Estimation of Sitagliptin phosphate and Metformin hydrochloride in Bulk and Tablet Dosage Form. *Der Pharm Chem* **4,** 854–859 (2012).

61. Nyola, N. & Jeyabalan, G. Method Development of Simultaneous Estimation of Sitagliptin and Metformin Hydrochloride In Pure And tablet Dosage Form By UV-Vis Spectroscopy. *World J Pharma and Pharm Sci* **4** (2012).

62. Riad, S. & Rezk, M. Spectrophotometric determination of sitagliptin and metformin in their pharmaceutical formulation. *Pharm. Glob.* **3**(5), 1-4 (2012).

63. Sharma, S., Kothari, A. & Harsoliya, M. Simultaneous UV Spectrophotometric Method for Estimation of Sitagliptin Phosphate and Simvastatin in Tablet dosage Form. *J. Pharm. Res.* **5**(1), 444-446 (2012).

64. Bawane, V., Sable, P. & Siddiqui, A. Uv Spectroscopic Simultaneous Estimation Of Sitagliptin Phosphate And Metformin Hydrochloride In Bulk And In Dosage Form. *Inven. Rapid Pharm Anal. Qual.* **4**, 1197 (2013).

65. Chavhan, V., Nigade, S. & Patel, J. Development and validation of economic UV spectrophotometric method for simultaneous estimation of Sitagliptin phosphate and Simvas-tatin in bulk and tablet dosage form by absorption ratio method. *J Pharm Phytother.* **1**(6), 5-10 (2013).

66. PV, S., Phanindra, S. & Anasuya, P. Development and Validation of Simultaneous Equation Method for the Estimation of Metformin and Sitagliptin by UV Spectroscopy. *Res and Rev. J.Pharm. and pharma. Sci.* **2**(2), 72-75 (2013).

67. PV, S., Reddy, Y. & Devanna, N. New Validated Spectroscopic Method for the Simultaneous Estimation of Simvastatin and Sitagliptin. *Res and Rev. J.Pharm. and pharma. Sci.* **3**(1), 32-36 (2014).

68. Phaneemdra, D. Simultaneous estimation of simvastatin and sitagliptin by using different analytical methods. *Int. J. Adv. Pharma. Ana.* **2**(1), 19-23 (2012).

69. Patil, S., Ramesh, B., Hareesh, A., Patil, K. & Dhokane, A. Development and validation of RP-HPLC method for the estimation of sitagliptin phosphate in tablet dosage form. *Asian J. Res. Chem.* **3**(3), 653-655 (2010).

70. Maste, M., Parate, A. & Bhat, A. Validation and Application of a High-Performance Liquid Chromatography Method for Estimation of Sitagliptin Phosphate from Bulk Drug and Pharmaceutical Formulation. *Asian J. Res.Chem.* **4**(9), 1466-1468(2011).

71. Dubala, A., Khatwal, R. & Kosaraju, J. Bioanalytical method development and validation of sitagliptin phosphate by RP-HPLC and its application to pharmacokinetic study. *Int. J. Pharm. Pharm. Sci.* **4,** 4–7 (2012).

72. Gawande, V., Narvekar, S. & Rathor, P. Simple, Rapid RP-HPLC Method For Estimation Of Sitagliptin From Urine And Its Application In Pharmacokinetics. *Int. J. Bioassay* **2**(10), 1322-1326 (2013).

73. Deepthi, V. & Poornima, Y. STABILITY-INDICATING RPHPLC METHOD FOR ANALYSIS OF SITAGLIPTIN IN THE BULK DRUG AND IT'S PHARMACEUTICAL DOSAGE FORM. *Int. J.Pharm. Pharm. Sci.* **5**(1), 320-325 (2013).

74. Pulla, R. & Sastry, B. Simultaneous Estimation of Metformin HCl and Sitagliptin Phosphate in Tablet Dosage Forms by RP-HPLC. *Res. J. Pharm. Tech* **4**(4), 646-649 (2011).

75. Raja, T. & Rao, A. Validated RP-HPLC Method for Simultaneous Estimation of Metformin Hydrochloride and Sitagliptin Phosphate in Bulk Drug and Pharmaceutical Formulation. *Int. J. Pharm. Chem. Bio. Sci.* **2**(4), 696-702 (2012).

76. Bhende, S. & Varanasi, M. B. RP-HPLC Method for the Simultaneous Estimation of Sitagliptin Phosphate and Metformin Hydrochloride in Combined Tablet Dosage Forms. *Orient. J. Cheem.* **28** (1), 463-469 (2012).

77. Gebremriam Ketema*, D. G. S. Development And Validation Of RP-HPLC Method For Simultaneous Estimation Of Sitagliptin And Simvastatin In Bulk And Tablet Dosage Forms. *Inven. Impact Pharm Anal. Qual. Assur.* **278** (12), (2012).

78. Inamdar, H. & Mhaske, A. RP-HPLC method for simultaneous determination of Metformin Hydrochloride, Rosiglitazone and Sitagliptin–application to commercially available Drug. *Int J Pharm Sci Res* **3**(9), 3267-3276 (2012).

79. Jeyabalan, G. & Nyola, N. Simultaneous estimation of Sitagliptin phosphate monohydrate and metformin hydrochloride in bulk and pharmaceutical formulation by RP-HPLC. *J Pharm Educ Res* **3**(2), 24-28 (2012).

80. Loni, A. Method development and validation for simultaneous determination of sitagliptin phosphate and metformin hydrochloride by RP-HPLC in bulk and tablet. *Asian J Pharm Sci* **2**(8), 23-37 (2012).

81. Malleswararao, C. Simultaneous determination of sitagliptin phosphate monohydrate and metformin hydrochloride in tablets by a validated UPLC method. *Sci. Pharm.* **80** (1), 139-152 (2012).

82. Raghava, K. & Rao, S. Simultaneous estimation of Sitagliptin and Simvastatin in Tablet Dosage Form By A Validated RP-HPLC Method. *Int. J. Pharm. Sci. Rev. Res.* **15** (1), 41-44 (2012).

83. Sumithra, M., Shanmugasudaram, M. & Sankar, A. Development of RP-HPLC method and it's validation for simultaneous estimation of sitagliptin and Metformin. *Int. J. Pharm Chem Sci…* 1(1), 360-364 (2012).

84. Kumar, S. A., Debnath, M. & Rao, J. S. Development of stability indicating RP-HPLC method for simultaneous estimation of metformin hydrochloride and sitagliptin phosphate monohydrate in bulk as well as in pharmaceutical formulations. *Der Pharm. Sin.* **4** (4), 47-61 (2013).

85. Kumar, S., Debnath, M. & Rao, D. Simultaneous Estimation of Sitagliptin Phosphate Monohydrate and Metformin Hydrochloride in Bulk and Pharmaceutical Formulation by RP-HPLC. *Am. J. Pharm. Tech. Res.* **3** (3), 556-575 (2013).

86. Patil, S., Jayant, R. & Chetan, M. Development and Validation of a Stability Indicating RP-HPLC Method for Simultaneous Determination of Sitagliptin and Metformin in Tablet Dosage Form. *Pharma Biomed. Sci* **4**(2), 590-596 (2013).

87. Sirisha, G. R., Vasanth, P., Ramesh, T. & Ramesh, M. RP-HPLC method development and validation for simultaneous estimation of Metformin and Sitagliptin in tablet dosage forms. *Int J Pharm Res Ana* **3**(1), 8-12 (2013).

88. Vemula, L., Tamilselvi, N. & Krishnan, R. A validated RP-HPLC method for simultaneous estimation of sitagliptin and simvastatin in tablet dosage form. *Int J Pharm Pharm Sci* **5**(2), 429-431 (2013).

89. Veronica, B. N. Development and validation of a new simple RP-HPLC method for estimation of Metformin HCl and Sitagliptin phosphate simultaneously in bulk and dosage. *Int J Adv Pharm Gen Res* **2**(1), 1-14 (2013).

90. Vani, R., Kumer, B. & Mohan, G. Analytical Method Development And Validation For The Determination Of Sitagliptin And Metformin Using Reverse Phase HPLC. *World J Pharm & Pharm Sci* **3**(3), 1803-1811 (2014).

91. Juvvigunta, R., Reddy, G., Dhanalakshmi, D. & Ramesh, B. A New Analytical Method Development and Validation for Simultaneous Estimation of Sitagliptin and Metformin Hydrochloride in Tablet Dosage form by RP-HPLC. *Int J Pharma Sci.* **3**(5), 360-364 (2013).

92. PV, S., Reddy, Y., Devanna, N. & Phanindra, S. Validated RP-HPLC Method for the Estimation of Simvastatin and Sitagliptin. *Sch. Acad. J. Pharm.* 3(3), 265-270 (2014).

93. Reddy, G., Kumar, S. & Kumar, V. A New, Simple, Sensitive, Accurate & Rapid Analytical Method Development & Validation for Simultaneous Estimation of Sitagliptin & Simvastatin in Tablet. *Int. J. Pharm Tech Res.* **6**(3), 880-893 (2014).

94. Shirisha, B., Prathyusha, B. & Ramathilagam, N. Development and Validation of RP-HPLC Method for Simultaneous Estimation of Sitagliptin and Simvastatin in Bulk and Tablet Dosage Form. *Int. J. Pharm* and *Anal Res* **2**(4), 175-183 (2013).

95. Tushar, R. P., Tejas, B. P. & Bhanubhai, N. S. Stability Indicating RP-HPLC Method For Simultaneous Estimation Of Simvastatin And Sitagliptin In Tablet Dosage Form. Indo American Journal Of Pharmaceutical Research, **4** (4), 1993-1999 (2014).

96. Modi, D., Parejiya, P. & Patel, B. A Simple and Sensitive HPTLC Method for Simultaneous Determination of Metformin Hydrochloride and Sitagliptin Phosphate in Tablet Dosage Form. *J. Chem.* **2013**, 1-4 (2013).

97. Raja, T. & Rao, A. Validated HPTLC Method for Simultaneous Estimation of Metformin Hydrochloride and Sitagliptin Phosphate in Bulk Drug and Formulation. *Rasayan J. Chem.* **5**(3) 407-413 (2012).

98. Sonali, R., Pallavi, P. & Vittal, C. Development and Validation of HPTLC method for the estimation of Sitagliptin Phosphate and Simvastatin in bulk and Marketed Formulation. *Int. J. Drug Dev. Res* **4,** 292–297 (2012).

99. Patel, C., Motisariya, M. & Shah, P. Validated HPTLC method for the simultaneous determination of metformin hydrochloride and sitagliptin phosphate in marketed formulation. *Int J Anal Bioanal Chem* **3**(1) 47-51 (2013).

100. Barge, V. & Warghade, S. Validated HPTLC Method for Simultaneous Estimation of Sitagliptin and Metformin Hydrochloride in Bulk Drug and Formulation. *Res. Rev. J. Pharm. Anal.* **3**(3) 45-50 (2014).

101. Salim, M., El-Enany, N. & Belal, F. Simultaneous determination of sitagliptin and metformin in pharmaceutical preparations by capillary zone electrophoresis and its application to human plasma. *Anal. Chem. Insights* **7**, 31-36 (2012).

102. Sharma, S. Developement of UV SPectrophotometry and RP HPLC Method and Its Validation for Simultaneous Estimation of Sitagliptin Phosphate and Simvastatin in Marketed Formulation. *Int. J. Pharm. Biol. Arch.* **3,** 673–678 (2012).

103. Jadhav, S., Kupkar, S. & Dharam, D. Development and Validation of RP-HPLC and HPTLC Methods for Simultaneous Estimation of Sitagliptin Phosphate and Metformin Hydrochloride in Bulk and. *Ind J Pharm Edu Res* **47** (1), 13-16 (2013).

104. Reddy, B. V. R. & Raman, N. Chiral separation of sitagliptin phosphate enantiomer by HPLC using amylose based chiral stationary phase. *J. Pharm. Res.* **7**(6), 546-550 (2013).

Submitted by	**Forwarded by**
Mr. Kunjan Bodiwala **Ph.D. Student**	**Dr. S. A. Shah** **Supervising teacher for Ph. D. degree**